US5313947 9

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 545 911 A2**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **93200152.2**

(22) Date of filing: **16.06.89**

(51) Int. Cl.5: **A61K 37/02**, //(A61K37/02, 31:23)

This application was filed on 21 - 01 - 1993 as a divisional application to the application mentioned under INID code 60.

(30) Priority: **01.03.89 US 317626**
22.06.88 US 209861

(43) Date of publication of application:
09.06.93 Bulletin 93/23

(60) Publication number of the earlier application in accordance with Art.76 EPC: **0 382 814**

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(71) Applicant: **APPLIED MICROBIOLOGY, INC.**
170 53rd Street
Brooklyn, N.Y. 11232(US)

(72) Inventor: **Blackburn, Peter**
426 West 44th Street
New York, New York 10036(US)
Inventor: **Polak, June**
57 Montague Street
Brooklyn, New York 11201(US)
Inventor: **Gusik, Sara-Ann**
317 First Avenue
New York, New York 10003(US)
Inventor: **Rubino, Stephen D.**
97 Whitney Drive
Berkeley Heights, New Jersey 07922(US)

(74) Representative: **Lucas, Brian Ronald**
Lucas & Co. 135 Westhall Road
Warlingham Surrey CR6 9HJ (GB)

(54) **Lanthionine-containing bacteriocin compositions for use as bactericides.**

(57) Compositions comprising a lanthionine-containing bacteriocin such as nisin and a surfactant, especially a nonionic one, are useful as bactericides, especially against Gram positive bacteria. They have both food and medicinal applications. To avoid overlap of Claim with the parent application, chelating agents are excluded from the claimed compositions and uses.

EP 0 545 911 A2

Nisin is a polypeptide with antimicrobial properties which is produced in nature by various strains of the bacterium Streptococcus lactis. It is a known food preservative which inhibits the outgrowth of spores of certain species of Gram positive Bacilli.

Although sometimes mistakenly and imprecisely referred to as an antibiotic, nisin is more correctly classified as a bacteriocin, i.e., a proteinaceous substance produced by bacteria and which has antibacterial activity only towards species closely related to the species of its origin. Nisin is a naturally-occurring preservative found in low concentration in milk and cheese, and is believed to be completely non-toxic and non-allergenic to humans.

Nisin has recently been recognized as safe by the FDA as a direct food ingredient in pasteurized cheese spread, pasteurized processed cheese spread, and pasteurized or pasteurized processed cheese spread with fruits, vegetables, or meats. Furthermore, since it is a polypeptide, any nisin residues remaining in foods are quickly digested.

A summary of nisin's properties appears in Hurst, A., Advances in Applied Microbiology 27:85-123 (1981). This publication describes what is generally known about nisin. Nisin, produced by Streptococcus lactis, is available commercially as an impure preparation, Nisaplin™, from Aplin & Barrett Ltd., Dorset, England and can be obtained by isolating naturally-occurring nisin from cultures of Streptococcus lactis and then concentrating the nisin according to known methods. There are also reported methods for producing nisin using altered strains of Streptococcus. See Gonzalez et al., US Pat. No. 4,716,115, issued December 29, 1987. It should also be possible to produce nisin by recombinant DNA technology.

Nisin has been applied effectively as a preservative in dairy products, such as processed cheese, cream and milk. The use of nisin in processed cheese products has been the subject of recent patents. See US Pat. Nos. 4,584,199 and 4,597,972. The use of nisin to inhibit the growth of certain Gram positive bacteria has been well documented. However, its complete success and acceptance as a food preservative has heretofore been hampered by the belief that nisin was ineffective against Gram negative and many Gram positive bacteria. Gram negative bacteria are almost always present in conjunction with Gram positive bacteria and are a major source of food spoilage and contamination. See Taylor, US Pat. No. 5,584,199, issued April 22, 1986 and Taylor, US Pat. No. 4,597,972, issued July 1, 1986; Tsai and Sandine, "Conjugal Transfer of Nisin Plasmid Genes from Streptococcus Lactis 7962 to Leuconostoc Dextranicum 181, Applied and Environmental Microbiology, Feb. 1987, p. 352; "A Natural Preservative", Food Engineering Int'l, May 1987, pp. 37-38; "Focus on Nisin", Food Manufacture, March 1987, p. 63.

It has now been found that in the presence of surfactant alone, nisin has enhanced activity against Gram positive bacteria.

The parent application relates to compositions of enhanced bactericidal activity comprising a lanthionine-containing bacteriocin and a chelating agent. These compositions can also contain a surfactant.

It has now been found that in the presence of surfactant alone, nisin has enhanced activity against Gram positive bacteria.

This invention provides bacteriocin compositions of nisin or other, lanthionine containing bacteriocins, in combination with surfactants. The invention further provides the compositions dissolved or suspended in a suitable carrier to yield enhanced broad range bactericides.

In the present invention, surfactants, valuable as cleansing agents, suitable for combination with nisin include, but are not limited to, the nonionic surfactants Tweens, Tritons, and glycerides, ionic surfactants such as fatty acids, quaternary compounds, anionic surfactants and amphoteric surfactants such as cocamidopropyl betaine and emulsifiers.

Since Gram positive and Gram negative bacteria are almost always found together in foods, the effectiveness of the nisin compositions towards Gram negative bacteria such as Salmonella typhimurium, Escherichia Coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacterioides gingivalis, Actinobacillus actinomycetescomitans, and other Gram negative pathogens and Gram positive bacteria will be of great use. The bactericides are particularly suited for the control and prevention of contamination of raw ingredients, processed foods and beverages by bacterial pathogens and other microbial spoilage organisms. Potential food related uses include treatment of meats, especially poultry, eggs, cheese and fish and treatment of food packaging and handling equipment. Further uses include as food preservative, such as in processed cheese, cream, milk, dairy products and in cleaning poultry, fish, meats, vegetables, and dairy and food processing equipment. The use of the nisin compositions should not be limited to food related used and the nisin compositions should be useful in any situation in which there is a need or desire to eliminate Gram negative and Gram positive bacteria.

The compositions can be dissolved in a suitable carrier for example an aqueous solvent or buffer or suspended in any suitable liquid, colloidal or polymeric matrix to create bactericides. Such compositions preferably contain from 0.01 to 0.2 percent of the surfactant. The compositions or bactericides can be

Nisin Bactericidal Activity towards
Streptococcus agalactiae in milk at 37°C

(Activation of nisin by monolaurin)

Nisin (μ g/ml)	Monolaurin (%)		
	0	0.01	0.1
	% survival at 2hr[a]		
0	100	100	4.5
0.02	100	100	0.2
0.2	2.2	0.05	0.0008

[a] Initial viable counts 6.0×10^7 cfu/ml.
Incubations were in milk at 37°C.

Example 2

Nisin and Surfactant (glyceride monooleate) Activity Against Gram positive Bacteria

An example of where the application of nisin is limited by its available activity is illustrated by the data in Table B. Although nisin is bactericidal towards L. monocytogenes, the data in Table B demonstrate that in a complex medium like milk the available nisin activity towards this organism is restricted. However, the bactericide comprised of nisin with the glyceride monooleate is effective in milk towards this foodborne pathogen, even though monooleate by itself had no bactericidal activity towards this organism.

Table B

Nisin Bactericidal Activity towards Listeria monocytogenes in milk at 37°C

(Activation of nisin by monooleate)

Nisin (μ g/ml)	% Monooleate 0	0.1	1.0
	% survival at 2hr[a]		
0	100	67	63
100	0.56	10^{-3}	10^{-4}

[a] Initial viable count 5.0×10^7 cfu/ml. Incubations were in milk at 37°C.

Claims

1. A bactericidal composition comprising a lanthionine-containing bacteriocin, characterized in that it includes a surfactant, but excludes a chelating agent.

2. A composition according to Claim 1, characterized in that the surfactant is non-ionic.

3. A composition according to Claim 1, characterized in that the surfactant is monolaurin or glyceride monooleate.

4. A composition according to Claim 1, characterized in that the surfactant is selected from the group consisting of Tritons, Tweens, glycerides, fatty acids, emulsifiers, quaternary compounds, amphoteric and anionic surfactants.

5. A composition according to any preceding Claim, characterized in that the lanthionine-containing bacteriocin is nisin or subtilin.

6. A composition according to any preceding Claim, characterized in that it further comprises a carrier.

7. A composition according to Claim 6, characterized in that the concentration of surfactant is from 0.01 to 1.0 percent.

8. A composition according to Claim 6 or 7, characterized in that the carrier is an aqueous solvent or buffer.

9. Use, other than in surgery or therapy, in combination of (1) a lanthionine-containing bacteriocin and (2) a surfactant, in the absence of a chelating agent, as a bactericide effective against Gram positive

bacteria.

10. Use according to Claim 9 for preserving food.

11. Use according to Claim 10 for preserving dairy products.

12. Use according to Claim 11 for preserving milk.

13. Use according to Claim 9 for cleaning poultry, fish, meats vegetables and dairy and food processing equipment.

14. A composition according to any one of Claims 1 to 8 for medicinal use.

15. A composition according to Claim 14 for use in the treatment of infections, wound dressings or surgical implants.

16. A composition according to Claim 14 for use as a broad spectrum disinfectant for skin or oral rinses, disinfectant scrubs, wipes or lotions.

17. An enhanced broad range bactericide comprising a carrier, a lanthionine-containing bacteriocin and a surfactant, but not containing a chelating agent.

18. A bactericide according to Claim 17, wherein the surfactant is selected from the group consisting of Tritons, Tweens, glycerides, fatty acids, emulsifiers, quaternary compounds, amphoteric and anionic surfactants and is present in an amount sufficient such that the bactericide has enhanced effectiveness against at least one of the bacteria from the group consisting of Gram negative and Gram positive bacteria.

19. A bactericide according to Claim 18, wherein the concentration of surfactant is between about 0.01 and 1.0 percent.

20. Each and every portion of the description and Claims of the parent Application No. 89 907 595.6 insofar as relates to compositions comprising a lanthioninecontaining bacteriocin and a surfactant or the conjoint use of said bacteriocin and surfactant as a bactericide, but excluding subject matter claimed in the allowed Claims of said application.